USDA-ARS | U.S. Wheat and Barley Scab Initiative

FY21 Performance Progress Report

Due date: July 26, 2022

Cover Page

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FY20 USDA-ARS Award Amount:	$60,343
Recipient Organization:	Purdue University Department of Botany and Plant Pathology Lilly Hall, 915 W State St. West Lafayette, IN 47907
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Reporting Period End Date:	5/31/2022

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
PBG	Mechanisms of Ammonium Sensing and Ammonium Suppression of DON Biosynthesis	$60,343
	FY21 Total ARS Award Amount	$60,343

I am submitting this report as an: ☒ Annual Report ☐ Final Report

I certify to the best of my knowledge and belief that this report is correct and complete for performance of activities for the purposes set forth in the award documents.

Principal Investigator Signature

7/25/2022

Date Report Submitted

‡ BAR-CP – Barley Coordinated Project
DUR-CP – Durum Coordinated Project
EC-HQ – Executive Committee-Headquarters
FST-R – Food Safety & Toxicology (Research)
FST-S – Food Safety & Toxicology (Service)
GDER – Gene Discovery & Engineering Resistance
HWW-CP – Hard Winter Wheat Coordinated Project
MGMT – FHB Management
MGMT-IM – FHB Management – Integrated Management Coordinated Project
PBG – Pathogen Biology & Genetics
TSCI – Transformational Science
VDHR – Variety Development & Uniform Nurseries
NWW –Northern Soft Winter Wheat Region
SPR – Spring Wheat Region
SWW – Southern Soft Red Winter Wheat Region

Project 1: Mechanisms of Ammonium Sensing and Ammonium Suppression of DON Biosynthesis

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1. **What are the major goals and objectives of the research project?**
   The goal of this study is to understand how ammonium sensing leads to the suppression of DON production. Objective 1 aims to identify and characterize the amino acid sequences of Mep2-CT responsible for ammonium suppression of DON production. The roles of Mep2 in regulating Are1 and genes responsible for the uptake/utilization of arginine or putrescine also will be determined. Objective 2 aims to characterize the interaction and functional relationship between Mep2 and Ras2 and the roles of cAMP-PKA in ammonium repression. Objective 3 will determine the roles of Are1 in *TRI* gene expression and Mep2 functions.

2. **What was accomplished under these goals or objectives?** *(For each major goal/objective, address these three items below.)*

   a) What were the major activities?

   Objective 1. We generated chimeric alleles of *MEP2* with parts of its sequence replaced with that of *MEP3* and identified the region conferring MEP2-specific functions as the ammonium sensor. Further analysis indicated that amino acid residues A^{480} and T^{493} play critical roles in Mep2 functions. We also showed that Mep2 and Are1 are involved in the regulation of the *FgCAR1*, *FgDUR3*, and *FgAGP2* genes. Deletion of both *MEP2* and *MEP3* affected ascospore releasing and resulted in more severe defects in conidium germination and hyphal growth under low ammonium conditions.

   Objective 2. Transformants of *mep2* mutant expressing the dominant active $RAS2^{DA}$ and dominant negative $RAS2^{DN}$ alleles were generated. Only the *mep2* $RAS2^{DA}$ transformants were partially rescued in conidium germination and growth defects and ammonium suppression of DON biosynthesis. Expression of $RAS2^{DA}$ increased PKA activities and Pmk1 phosphorylation in *mep2* mutant and rescued its defects in ammonium suppression of *TRI* genes. We also conducted yeast two-hybrid, BiFC, and co-IP assays but failed to detect the interaction between Ras2 and cytoplasmic tail of Mep2. We also found that the *mep2* mutant was significantly reduced in the expression level of *ARE1*, which is functionally related to PKA and Gpmk1 kinases and involved in regulating DON biosynthesis

   Objective 3. We used strong constitutive RP27 and RP30 promoters to express Are1-Flag and Are1-S-tag but failed to detect these fusion proteins on western blots. (It is likely that Are1 is unstable or expressed at a very low level) Because it is impossible for ChIP-seq and phosphoproteomics assays, as alternative approaches, we deleted two predicted Are1-binding sites in the *TRI5* promoter. In the resulting transformants, *TRI5* expression was reduced but ammonium suppression on *TRI5* was diminished. We also mutated predicted PKA and MAPK phosphorylation sites in *ARE1* and found that mutations at individual sites had no significantly effects on its function, likely due to phosphorylation at multiple sites

   b) What were the significant results?

   Objective 1. The first 39 amino acid residues of the C-terminal cytoplasmic region of Mep2, particularly A^{480} and T^{493}, were found to play important roles in Mep2-specific function as the ammonium sensor to regulate DON biosynthesis. We also showed the involvement of Mep2 and Are1 in regulating genes related to the uptake or metabolism of arginine and

putrescine that are known to induce DON production. *MEP2* and *MEP3* were found to have overlapping functions in conidium germination and hyphal growth under ammonium limitation conditions as well as ascospore discharge, which is important for plant infection. **Objective 2**. We showed that Ras2 functions downstream from Mep2 ammonium sensor to regulate DON production, likely via the cAMP-PKA and MAPK signal transduction pathways in response to nitrogen starvation signals in *F. graminearum*. Expressing the dominant active $RAS2^{DA}$ rescued the defects of *mep2* mutant in ammonium suppression of *ARE1* transcription factor, genes involved in nitrate metabolism, and DON biosynthesis. **Objective 3**. Whereas objectives 1 and 2 are the main objectives to characterize the roles of Mep2 as an ammonium sensor and its functional relationship with Ras2 signaling to regulate DON production, objective 3 is to characterize the functional relationship between Are1 and Mep2 on regulating *TRI* gene expression. Although we had difficulties to conduct ChIP-seq and phosphoproteomics assays, we characterized the functions of predicted Are1-binding sites in *TRI5* and predicted PKA or MAPK phosphorylation sites in Are1

c) List key outcomes or other achievements.

Objective 1.

- Identified the region and amino acid residues that are essential for the Mep2-specific functions as an ammonium sensor for regulating DON biosynthesis.
- Characterized the roles of Mep2 and Are1 in regulating genes related to the uptake or metabolism of arginine and putrescine that are inducive to DON production.
- Identified the overlapping functions between Mep2 and Mep3 in conidium germination and ascospore discharge.

Objective 2.

- Demonstrated the role of Ras2 in intracellular signaling downstream from Mep2 ammonium sensor for regulating TRI gene expression and DON production.
- Showed the requirement of Mep2 in ammonium suppression of genes involved in nitrate metabolism and DON biosynthesis.

Objective 3.

- Determined the importance of the putative Are1-binding sites in the TRI5 promoter.
- Characterized the predicted PKA and MAPK phosphorylation sites of Are1

3. What opportunities for training and professional development has the project provided?

One MS student (graduated in May, 2022), two PhD students, and a visiting student were involved in various aspects of this project. They were trained in different molecular techniques, including fungal transformation, qRT-PCR, western blot analysis, site-directed mutagenesis, and RNA-seq data analysis. They were also trained in preparing posters and Powerpoint presentations as well as manuscripts for publications.

4. How have the results been disseminated to communities of interest?

Results from our studies with the wheat scab fungus *Fusarium graminearum* have been published in referred scientific journals and a book chapter, and presented at conferences attached by scientists with interests in DON and this disease. I also include data from this study in my lectures at 2022 Fusarium Workshop held as Kansas State University in June 2022.

Publications, Conference Papers, and Presentations

Please include a listing of all your publications/presentations about your FHB work that were a result of funding from your FY21 grant award. Only citations for publications published (submitted or accepted) or presentations presented during the **award period** should be included.

Did you publish/submit or present anything during this award period?

☒ Yes, I've included the citation reference in listing(s) below.
☐ No, I have nothing to report.

Journal publications as a result of FY21 grant award

List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Include any peer-reviewed publication in the periodically published proceedings of a scientific society, a conference, or the like.

Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published [include DOI#]; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

1) Hu, Y., Hou, R., Wang, Z.Y., Zhang, W. W., and Xu, J. R. Nitrogen repression of DON biosynthesis is mediated by Mep2 ammonium permease in *Fusarium graminearum*. 2022. *Environmental Microbiology*. Submitted; acknowledgment of federal support - yes.
2) Xu, H., Ye, M., Xia, A., Jiang, H., Huang, P., Liu, H., Hou, R., Li, D., Wang, Q., Xu, J. R., and Jiang, C. 2022. The Fng3 ING protein regulates H3 acetylation and H4 deacetylation by interacting with two distinct histone modifying complexes. *New Phytologist.* doi: 10.1111/nph.18294; acknowledgment of federal support - yes.
3) Lu, P., Chen, D., Qi, Z., Wang, H., Chen, Y., Wang, Q., Jiang, C., Xu, J. -R., and Liu, H. 2022. Landscape and regulation of alternative splicing and alternative polyadenylation in a plant pathogenic fungus. *New Phytologist.* doi: 10.1111/nph.18164; acknowledgment of federal support - yes.
4) Ren, J. Y., Zhang, Y., Wang, Y., Li, C.L., Bian, Z.Y., Zhang, Xu., Liu, H., Xu, J. R.*, and Jiang, C*. 2022. Deletion of all three MAP kinase genes results in severe defects in stress responses and pathogenesis in *Fusarium graminearum*. *Stress Biology*. 2 (1): 1-13. https://doi.org/10.1007/s44154-021-00025-y; acknowledgment of federal support - yes.
5) Li, C. H., Fan, Z., Huang, X., Wang, Q. H., Jiang, C., Xu, J. R., and Jin, Q. J. 2022. Mutations in FgPrp6 suppressive to the *Fgprp4* mutant in *Fusarium graminearum*. *Journal of Integrated Agriculture*. 21 (5): 1375-1388. https://doi.org/10.1016/S2095-3119(21)63731-0; acknowledgment of federal support - no.
6) Ye, M., Jiang, H., Fu, X,. Xu, J. R., and Jiang, C. 2021. Fng1 is involved in crosstalk between histone acetylation and methylation. *Current Genetics*. 67: 535-538. https://doi.org/10.1007/s00294-021-01167-2; acknowledgment of federal support - no.

Books or other non-periodical, one-time publications as a result of FY21 grant award

Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like.

Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (book, thesis or dissertation, other); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

1) Wang, Z.Y., Zhang, X., Jiang, C., and Xu, J. R. 2022. Regulation of plant infection processes by MAP kinase pathways in ascomycetous pathogens. In Mycota IX: Fungal Associations. Ed. M. Blackwell and Y. P. Hsueh. *Springer Nature*, Switzerland. Acknowledgement of federal support - no.

Other publications, conference papers and presentations as a result of FY21 grant award

Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication.

None